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RESEARCH NOTE

THE ACUTE AND CHRONIC TOXICITY OF THE DISPERSANTS COREXIT 9527 AND 9500, WATER ACCOMMODATED FRACTION (WAF) OF CRUDE OIL, AND DISPERSANT ENHANCED WAF (DEWAF) TO *HYDRA VIRIDISSIMA* (GREEN HYDRA)

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Abstract—The acute and chronic toxicities of the dispersants Corexit 9527 and Corexit 9500 to green hydra (*Hydra viridissima*) were determined. The mean (SE) 96 h LC50 values for Corexit 9527 and Corexit 9500 were 230 (4.8) ppm and 160 (2.3) ppm. The 7-day no-observed-effect-concentration (NOEC) and lowest-observed-effect-concentration (LOEC) values based on population growth rates were <15 and 15 ppm for Corexit 9527 and 13 and 43 ppm for Corexit 9500. The mean (SE) 96 h LC50 values for water accommodated fraction (WAF) of Bass Strait crude oil, Corexit 9527 dispersant enhanced WAF (DEWAF) and Corexit 9500 DEWAF were 0.7 (0.1) ppm total petroleum hydrocarbons (TPH), 9.0 (0.5) and 7.2 (0.1) ppm TPH. The NOEC and LOEC values based on 7-day population growth rates were: 0.6 and >0.6 ppm TPH for WAF, <0.6 and 0.6 ppm TPH for Corexit 9527 DEWAF, and 2 and 4 ppm TPH for Corexit 9500 DEWAF. © 1999 Elsevier Science Ltd. All rights reserved

Key words—hydra, toxicity, oil, dispersant, water accommodated fraction

INTRODUCTION

Few studies of oil toxicity have been conducted with freshwater organisms. Freshwater oil spills are often complicated by closer shorelines, greater water currents, smaller volumes of water, unidirectional flow and greater potential for floods than those occurring in the marine environment (Hayward Walker *et al.*, 1995). Dispersants are often recommended for treatment of freshwater oil spills. This approach may reduce the toxicity of spilled oil to surface animals such as birds and mammals. However, dispersing the oil may increase its toxicity to freshwater organisms, and the dispersant itself may be toxic (Vindimian *et al.*, 1992).

Two dispersants which have been used in freshwater situations are Corexit 9527 and Corexit 9500. Corexit 9527 contains the hydrocarbon solvent eth-

ylene glycol monobutyl ether (17%), and both non-ionic (48%) and anionic (35%) surfactants (Singer *et al.*, 1991; Exxon Chemical Australia Ltd, 1992). Corexit 9500 is a more recent formulation which provides enhanced penetration and emulsion fighting properties with the same surfactants as Corexit 9527.

The rapid rate of asexual reproduction of hydra (Cnidaria:Hydrozoa) by budding allows the population reproduction effects of a potential toxicant to be determined in the laboratory. Such chronic toxicity bioassays provide a rapid, sensitive and precise approach to the measurement of environmental pollutant effects on freshwater invertebrates (Stebbing and Pomroy, 1978). Hydra species have been shown to be sensitive to both metal and organic contaminants (Slooff *et al.*, 1983; Hyne *et al.*, 1992; Pollino and Holdway, in press).

The aims of this research were to use green hydra to assess the acute (mortality) and chronic (population growth) toxicity of the dispersants Corexit 9527 and Corexit 9500, Bass Strait crude oil water

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accommodated fraction (WAF), and mixtures of dispersant and crude oil WAF (Dispersant Enhanced WAF). The reference toxicant 4-chlorophenol was used to determine changes in the sensitivity of hydra over time (Environment Canada, 1990).

MATERIALS AND METHODS

Culture maintenance

Green hydra (*H. viridissima*) stock cultures were maintained in one litre glass bowls filled with autoclaved carbon-filtered mains water and located in a 25°C constant temperature room with 12-h light:dark photoperiod. Excess newly hatched *Artemia salina* nauplii (brine shrimp) were fed to hydra daily. Water was replaced after 30 min, and hydra were placed in clean bowls weekly.

Experimental design

Methods for acute toxicity testing were based on the Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians (E729-88a) (American Society for Testing and Materials, 1996). Chronic toxicity testing methods were based on the Hydra Test protocol of the Supervising Scientist Report (110) (Hyne *et al.*, 1996). Temperature, pH, dissolved oxygen and total hardness were measured for each test.

Acute toxicity tests

Twenty non-budding hydra were randomly assigned to glass Petri dishes and positioned randomly under the light. Concentrations were based on the results of range finder tests. The number of hydra in each Petri dish were counted at 0, 1, 2, 4, 8, 12, 24, 36, 48, 72 and 96 h. Hydra were not fed during the acute tests. During all tests hydra with unseparated buds were recorded, but counted as one hydra. The lethal toxicity end-points were the tulip and disintegrated stages. Each acute toxicity test series was performed four times using freshly prepared stock solutions.

Reference toxicant

4-Chlorophenol (Sigma, 99+ % purity) tests were static because of the highly stable nature in solution (Environment Canada, 1990). Test solution samples were taken at 0 and 96 hours for concentration analysis using the 'Direct Photometric Method for Phenols' in the Standard Methods for the Examination of Water and Wastewater (Greenberg *et al.*, 1992). Measured concentrations ranged from 0 to 80 (1.5) ppm 4-chlorophenol. A concentration close to the calculated LC50 for 4-chlorophenol (36 ppm) was run simultaneously with all other toxicity tests to determine if there was change in green hydra sensitivity over time.

Chronic toxicity tests

Each experiment was simultaneously replicated four times at a range of concentrations based on range finder tests. Five budding hydra were randomly assigned to each Petri dish, and the number of hydra were counted at 24 h, then daily for a total of 7 days. Hydra were fed to excess daily with 500 µl of live brine shrimp in test solution. After 30 min, test solutions were replaced. Water samples were collected on days 0, 4 and 7 for chemical analysis.

Corexit 9527 and Corexit 9500

Mean (SE) measured concentrations for acute tests ranged from 0 to 280 (8.0) ppm Corexit 9527 and 0 to 450 (19.6) ppm Corexit 9500. Mean (SE) chronic test measured

concentrations ranged from 0 to 320 (3.8) ppm Corexit 9527 and 0 to 170 (7.2) ppm Corexit 9500. Test solutions for concentration analysis were collected at 0 and 24 h, before and after solution replacement. Samples were measured at 230 nm in a UV-VIS spectrophotometer (Hitachi U2000).

WAF of crude oil

Crude oil and carbon-filtered mains water were mixed at a ratio of 1:9 (750 ml:6.75 l) in a 10-l glass mixing chamber (Fucik *et al.* 1995). The contents were mixed for 23 h in the dark with 1 h for settling. The aqueous layer (WAF) was used as the stock solution. Measured concentrations ranged from 0 to 1 ppm TPH for acute toxicity tests, and 0 to 0.6 ppm TPH for chronic tests. A 200-ml sample of WAF stock solution was extracted in hexane. GC analyses measured polycyclic aromatic hydrocarbons (PAH) and total petroleum hydrocarbons (TPH).

A time dependent toxicity test was performed to further observe hydra population growth rates. Five hydra were randomly placed in Petri dishes containing 1 ppm TPH for 0, 5, 10, 24, 30, 48 and 72 h and fed daily. After the appropriate time, hydra were pipetted into a Petri dish with carbon-filtered mains water until day 7. The controls were also placed in carbon-filtered mains water after 24 h.

A 200-ml sample of WAF stock solution was extracted in hexane, and total polycyclic aromatic hydrocarbons (PAH) and petroleum hydrocarbons (TPH) were measured by GC.

Corexit 9527 dispersant enhanced WAF (DEWAF) and Corexit 9500 DEWAF

WAF was prepared as previously described. Dispersant was mixed with crude oil collected from the top of the WAF mixing chamber at a ratio of 1:29 (3.45 ml:100 ml) as recommended by Exxon Chemical Australia Ltd (1992). This solution was mixed by hand for approximately 2 min, then 1 ml of this dispersant/oil solution was added to 1 l of WAF in a glass mixing bottle and mixed on a magnetic stirrer for 20 minutes. The aqueous layer was used as the stock solution. Measured concentrations for acute toxicity tests ranged from 0 to 20 ppm TPH for Corexit 9527 DEWAF and 0 to 10 ppm TPH for Corexit 9500 DEWAF. Measured concentrations for chronic toxicity tests ranged from 0 to 10 ppm TPH for Corexit 9527 DEWAF and 0 to 9 ppm TPH for Corexit 9500 DEWAF. The stock solution was analysed for PAH and TPH as described for WAF.

Analysis of results

The trimmed Spearman-Kärber method (Hamilton *et al.*, 1977) was used to calculate the 96 h LC50 values. *T*-tests were performed to detect significance between LC50 values within species.

For chronic toxicity tests, a one way analysis of variance (ANOVA) was used to determine if there was significant difference between the number of hydra on day 7 and the mean population growth rate (*K*) for different treatments. If the treatments were significant ($P \leq 0.05$), the Fisher's Least Significant Difference test ($P \leq 0.05$) determined which concentrations were significant. The mean population growth rate (*K*) on day 7 was calculated for all chronic toxicity tests. The mean population growth rate is defined as:

$$K(\text{number of hydra/day}) = \frac{\ln(ny) - \ln(nx)}{ty - tx}$$

where *ny* is the number of hydra on the final day, *nx* is the number of hydra on day 0 and *ty*–*tx* is the length of the experiment, in days.

Table 1. Mean (SE) 96 h LC50 values and 7-day population growth rate NOEC and LOEC values for green hydra ($n = 4$ for all toxicants) Values within LC50 column without a superscript in common are significantly different ($P \leq 0.05$)

Toxicant	LC50	NOEC	LOEC
4-chlorophenol (ppm)	34 (2.2) ^d	—	—
Corexit 9527 (ppm)	230 (4.8) ^f	< 15	15
Corexit 9500 (ppm)	160 (2.3) ^e	13	43
WAF (ppm TPH)	0.7 (0.1) ^a	0.6	> 0.6
Corexit 9527 DEWAF (ppm TPH)	9.0 (0.5) ^c	< 0.6	0.6
Corexit 9500 DEWAF (ppm TPH)	7.2 (0.1) ^b	2	4

RESULTS

Water characteristics

The range for pH was from 6.5 to 7.6, temperature 24.4 to 25.3°C, dissolved oxygen 8.3 to 8.8 ppm. All measurements including total water hardness did not vary significantly between experiments.

Reference toxicant

Green hydra exposed to 4-chlorophenol had a mean 96 h LC50 of 34 ppm (Table 1). Sensitivity to the reference toxicant did not differ significantly over the testing period (range 42–61% mortality).

Corexit 9527 and Corexit 9500

Corexit 9500 was more acutely toxic to green hydra than Corexit 9527 (Table 1). The mean population growth rates (K) of green hydra were reduced after 7 days of exposure, with Corexit 9500 being the more toxic dispersant (Fig. 1a).

WAF of crude oil

The WAF stock solution contained measured concentrations of 50 ppb PAH and 1 ppm TPH. WAF was highly toxic to green hydra in the short term (Table 1). Hydra growth rate did not decrease after chronic exposure to WAF concentrations tested. Population growth increased at the lower concentrations of WAF compared to the controls (Fig. 1b). Time dependent toxicity testing using 1 ppm TPH for 5–48 h showed a significant increase in hydra growth rate, and exposure for 72 h caused a significant decrease in hydra growth rate compared to the controls (Fig. 2).

Corexit 9527 DEWAF and Corexit 9500 DEWAF

The stock solutions contained measured concentrations of 100 ppb PAH and 20 ppm TPH for Corexit 9527 DEWAF, and 80 ppb PAH and 100 ppm TPH for Corexit 9500 DEWAF. Corexit 9500 DEWAF was more acutely toxic than Corexit 9527 DEWAF (Table 1). Corexit 9500 DEWAF reduced population growth rates at lower TPH concentrations than Corexit 9527 DEWAF (Fig. 1c).

DISCUSSION

The 4-chlorophenol 96 h LC50 of 34 ppm for green hydra in this study is comparable to the 96 h LC50 of 45 ppm previously reported for this lab-

oratory by Pollino and Holdway (in press). Hydra are less sensitive to acute 4-chlorophenol exposure than other test species, such as *Daphnia magna* with a 48 h LC50 of 6.5 ppm (Kuiper and Hanstveit, 1984).

It is unlikely green hydra will be affected by concentrations of dispersant used during oil spill treatment. Green hydra appear to be less sensitive to dispersants than other freshwater species, having a 96 h LC50 for Corexit 9527 of 230 ppm. This value is higher than *D. magna*, whose 96 h LC50 has been variably reported as 72.5 ppm (Shales, 1989, unpublished data) and 25 ppm (Brown and Goodman, 1989).

Previous researchers have used a variety of methods to assess the acute and chronic toxicity of crude oil WAF by preparing various water and oil combinations. This makes comparisons between animal species and oil types difficult. For example, Anderson *et al.* (1974) reported 96 h LC50 values for the brown shrimp (*Penaeus aztecus*) and grass shrimp (*P. pugio*) and 48 h LC50 values for the mysid (*Mysidopsis almyra*) for South Louisiana and Kuwait crude oils as greater than 6 ppm TPH in all cases. In comparison, exposure of green hydra to Bass Strait crude oil (WAF) showed greater sensitivity, with a 96 h LC50 of 0.7 ppm TPH.

Hydra growth rate increased after seven days of exposure to 0.1 and 0.3 ppm TPH of Bass Strait WAF compared to the controls. This increase in budding rate has been termed hormesis. Hormesis is an overcorrection of biosynthetic control mechanisms following exposure to low levels of contaminants, resulting in growth that is greater than normal (Stebbing, 1982). Hormesis has been observed previously in hydra exposed to low concentrations of lead (Browne and Davis, 1977) and copper (Pollino and Holdway, in press). Hormesis was also apparent after exposure of hydra to 1 ppm TPH for 48 hours or less. However, exposure of hydra to 1 ppm TPH for 72 hours caused the growth rate to fall below that of the control group.

Crude oil spilled into freshwater can be from burst pipelines or transport accidents. Aquatic organisms would be pulse-exposed to high concentrations of the product for a short period before spreading, evaporation, dissolution, and emulsification (Hayward Walker *et al.*, 1995). Continuous ex-

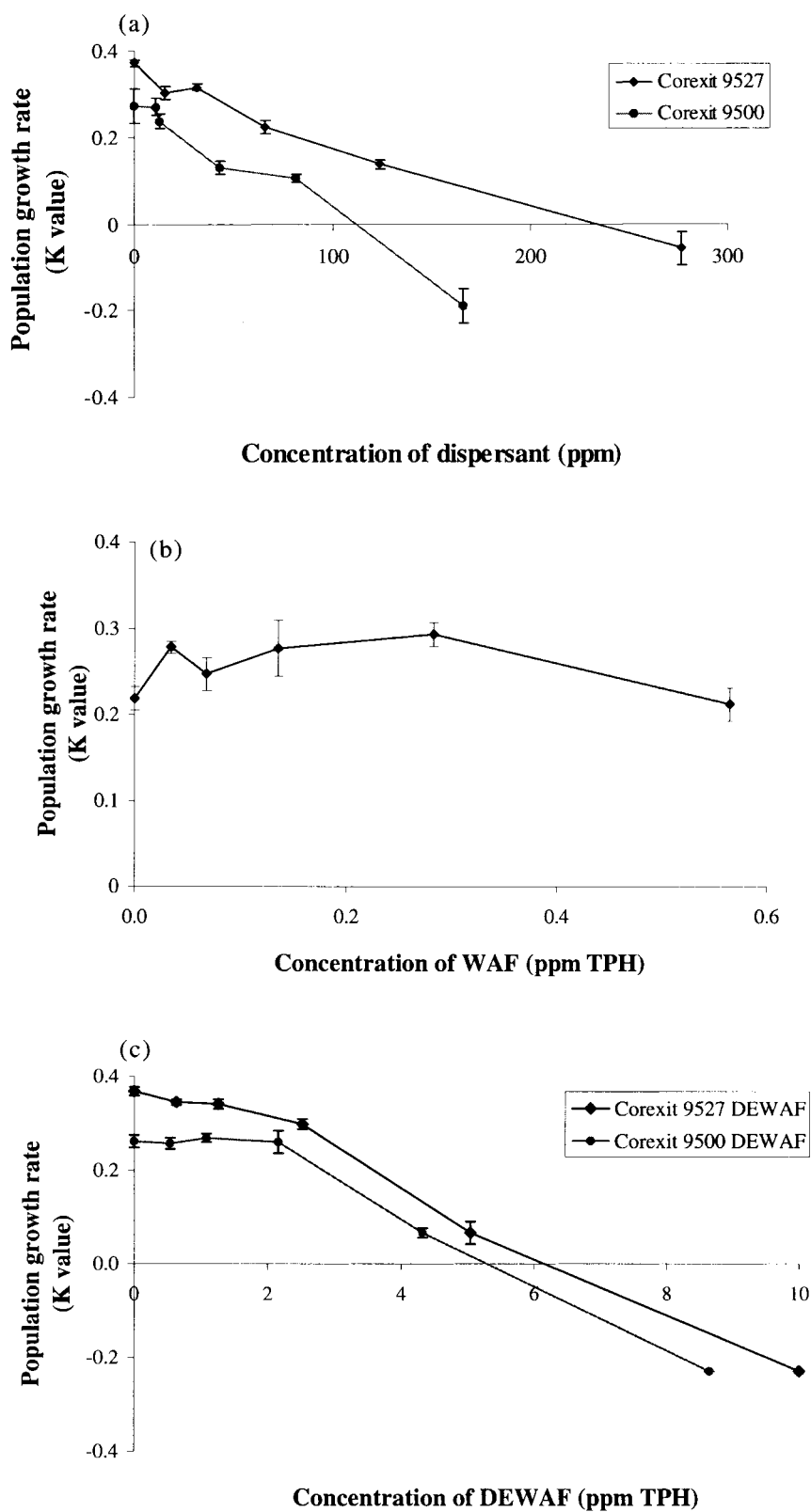


Fig. 1. Mean (SE) population growth rate of green hydra (number of hydra per day) after 7 days' exposure to (a) Corexit 9527 and Corexit 9500; (b) WAF; (c) Corexit 9527 DEWAF and Corexit 9500 DEWAF.

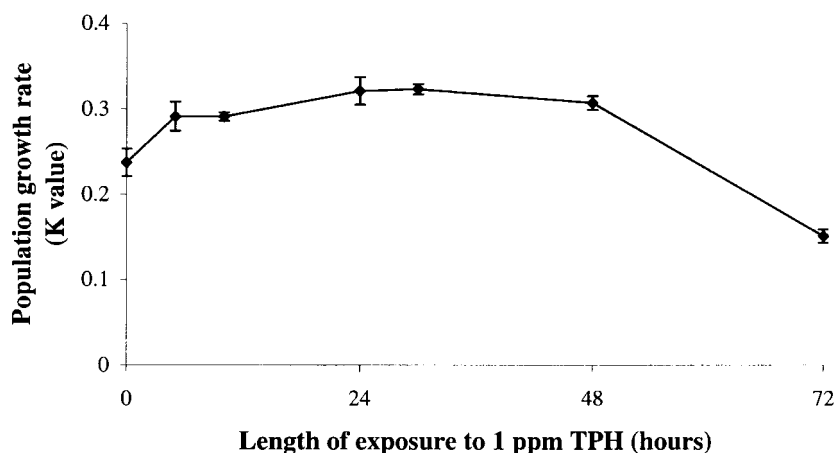


Fig. 2. Mean (SE) population 7 day growth rate of green hydra (number of hydra per day) after exposure to 1 ppm TPH for 0–3 days.

posure to WAF of crude oil for 96 h may therefore overestimate exposure.

Corexit 9500 DEWAF may have been more toxic to green hydra than Corexit 9527 DEWAF due to either the increased toxicity of the dispersant alone, or the toxic components of Bass Strait crude being more readily available to the hydra. By contrast, the chronic toxicity of Corexit 9527 DEWAF was greater than that of Corexit 9500 DEWAF. Perhaps Corexit 9527 DEWAF specifically affects budding processes.

The WAF of Bass Strait crude oil was more acutely toxic to green hydra than when tested in combination with either dispersant. The toxic components of the WAF may have been more readily available to hydra, either by ingestion or direct uptake. Both DEWAF stock solutions contained higher concentrations of both PAH and TPH; however, these components may not have been as readily available to the hydra.

WAF and DEWAF concentrations were near those expected in the environment following an oil spill, indicating that green hydra populations may be vulnerable to such events. It is unlikely that freshwater organisms in the environment would be continuously exposed to dispersants, WAF or DEWAF for the duration of toxicity tests conducted in the laboratory.

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